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October 28, 1994

Scientific Officer Code: 1141MB
Dr. Randall S. Alberte
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

Dear Dr. Alberte:

Three copies of the final technical report for our grant with the Office of Naval Research entitled "Biofilm Structure and Activity" (N00014-92-J-4011) are enclosed for your consideration. Copies of the report have been transmitted to the addresses indicated in the reports distribution list. I hope this meets with your satisfaction, and I would be pleased to provide additional information at your request.

Sincerely,

David A. Stahl
Associate Professor

Enclosure

cc: Administrative Grants Officer, Office of Naval Research
Director, Naval Research Laboratory
Defense Technical Information Center

FINAL TECHNICAL REPORT

Grant#: N00014-92-J-4011 **R&T Code: 3412-001**

PRINCIPAL INVESTIGATOR: David A. Stahl

GRANT TITLE: Biofilm Structure and Activity

AWARD PERIOD: 1 July 1992 - 30 June 1994

OBJECTIVES: To develop molecular measures of the identity, activity, and spatial distribution of microbial populations making up natural (multispecies) biofilms. To use biofilm bioreactors to characterize the relationship between biochemical transformations and community structure, focusing on the terminal steps of anaerobic mineralization and emphasizing competitive and cooperative interactions among sulfate-reducing and methanogenic biofilm populations. These studies and the development of new techniques should contribute to a better understanding of biofilm microbial populations and associated processes and, therefore, serve to provide a rational foundation for their control and manipulation.

APPROACH: Fixed-bed complete-mix bioreactor systems are used to study the structure and function of anaerobic biofilm communities using molecular and chemical measures in combination with mathematical modeling.

ACCOMPLISHMENTS:

Prevalence of Desulfovibrio in Sulfate-limited Biofilms. A major objective achieved during this project period was completion of a long term experiment characterizing biofilm response to addition and removal of sulfate. This experiment documented the persistence of a large and stable desulfovibrio population in biofilms deprived of sulfate.

An important component of the past research was the development of a mathematical model used to predict biofilm response to changing environment, including varying sulfate availability. The model is more fully described below (Modeling). The model is based on current understanding of the physiology of the major biofilm populations. Thus, an important result of our studies, as briefly described below, were observed deviations from model predictions. These indicated important syntrophic associations not included in the model and emphasized the value of structure/function studies to reveal interactions between biofilm populations.

Replicate reactors were established both in the presence and absence of sulfate (four reactors total). The replicates demonstrated comparable population makeup prior to perturbation. Selected experimental results of sulfate addition (to methanogenic biofilm reactors) or sulfate removal (from methanogenic reactors) are displayed in Figure 1. The biofilm community response was measured using the group-specific DNA probes for the sulfate-reducing bacteria and methanogens (developed during our ONR sponsored research) in combination with chemical analyses. The central observation was the persistence of high numbers of desulfovibrio in the absence of appreciable sulfate. Removal of sulfate from a long standing sulfidogenic biofilm system resulted in only an approximate 20 - 50% reduction in desulfovibrio abundance. Conversely, addition of sulfate to a long-standing (steady state) methanogenic biofilm resulted in the rapid onset of sulfate reduction (consistent with a standing population of sulfate reducing bacteria) and an approximate 30% increase in their abundance .

As further discussed below, the persistence of desulfovibrio in both the absence (17-20%) and presence (25-35%) of sulfate suggested a syntrophic association with a hydrogen consuming population in the absence of sulfate.

Desulfobacterium numbers (ca 5%) were not altered by changing sulfate availability. The desulfococcus group, present at about 2-3% in the absence of sulfate, was excluded from the biofilm following addition of sulfate. Thus, all three possible responses (increase, no change or decrease) were observed for different populations of sulfate-reducing bacteria following sulfate addition.

Similarly, the behavior of individual methanogenic groups was not captured by our model. The abundance's of *Methanosarcinaceae* (1-2%), *Methanococcales* (2-4%), and *Methanogenium*-relatives (4-5%) were little altered by sulfate availability. In contrast, the *Methanobacteriales* increased from approximately 1% to 15 - 17% of the biofilm community following sulfate removal, indicating they were in direct competition with the desulfovibrio for hydrogen.

The Role of Interspecies Hydrogen Transfer in Biofilm Processes. The persistence of sulfate-reducing bacteria in sulfate-limited biofilms implies an alternative metabolism in the absence of sulfate. They are either growing fermentatively and/or using an alternative electron acceptor. It is unlikely that added glucose is fermented by these desulfovibrio, since the *Desulfovibrio* spp. isolated from these systems (strain PT2) is unable to use sugars as a substrate, either fermentatively or coupled to sulfate reduction (Lau and Stahl, unpublished). An attractive hypothesis is that the desulfovibrio grow in the absence of sulfate via interspecies hydrogen transfer. A hydrogen-consuming population (e.g., a methanogen) allows substrate oxidation by the sulfate-reducing bacteria to be an energy liberating reaction by reducing the hydrogen concentration. The ability of certain desulfovibrio to grow in co-culture, as hydrogen-producing syntrophs, in the absence of sulfate is well established. However, the general environmental significance of this has not been well explored.

Although we predict that interspecies hydrogen transfer is most important to stabilizing the desulfovibrio in the absence of sulfate, the methanogens may not be the only relevant hydrogen consuming populations. This study also revealed that following removal of sulfate, the desulfovibrio population stabilized (at about 65% of the sulfate grown population size) before methanogen populations significantly increased (Figure 1). Thus, another hydrogen consuming population may be more important than methanogens during the initial period following sulfate removal. The other major group of hydrogenotrophic anaerobes are the homoacetogens (Dolfing 1988). The common substrate for this group is also H_2 and CO_2, yielding acetate (as opposed to methane). Although methane is the thermodynamically favored end-product, some natural anaerobic systems are primarily acetogenic, as opposed to methanogenic. The factors controlling this relationship are not yet understood.

Measurement of Single Cell Activity. We completed a study using cellular ribosome content as a basis to assess the activity of individual biofilm bacteria (Poulsen et al. 1993). The study population, a desulfovibrio species, was originally identified using 16S rRNA sequences directly recovered from reactor biofilms (Amann et al. 1992). The sequence information was used to design a species-specific DNA probe for microscopic studies of biofilm distribution and to screen enrichment cultures for use in isolation. The corresponding organism (strain PT2) was successfully isolated. Strain PT2 is most closely related to *Desulfovibrio vulgaris* (Hildenbourough). Physiological studies of strain PT2 were conducted to define its growth-rate-associated change in cellular ribosome content (Poulsen et al. 1993), as a prelude to studies of it's activity in reactor biofilms. Ribosome content was measured using a cooled CCD camera (Photometrics) to quantify fluorescence of single cells following hybridization with fluorescent-dye labeled DNA probes complementary to the 16S rRNA. PT2 cells associated with recently colonized surfaces were compared to those present in long-

established biofilm reactors. This revealed that the desulfovibrio associated with new biofilms were much more active than those in established biofilms (Poulsen et al 1993), and demonstrated the general utility of the method for in situ activity studies. This method will be used in future research to evaluate growth-related response to changing reactor conditions, activity differences associated with relative position in the biofilm, or conferred by specific associations with other biofilm populations.

Modeling. We developed and applied a mathematical model for the complex interactions among the key microbial groups in our anaerobic-biofilm reactors (Raskin 1993, Raskin et al. unpublished). Nine groups of microorganisms were represented:

1. Bacteria (previously Eubacteria) fermenting glucose to propionate, butyrate, acetate, and H_2.
2. Obligate proton-reducing bacteria that ferment propionate to acetate and H_2.
3. Obligate proton-reducing bacteria that ferment butyrate to acetate and H_2.
4. Acetoclastic methanogens.
5. H_2-oxidizing methanogens.
6. Incomplete propionate-oxidizing SRB, which produce acetate and H_2 from propionate while reducing sulfate.
7. Incomplete butyrate-oxidizing SRB, which produce acetate and H_2 from butyrate while reducing sulfate.
8. H_2-oxidizing SRB.
9. Acetate-oxidizing SRB.

The model explicitly considers all nine groups and their limiting substrates and catabolic products (i.e., glucose, propionate, butyrate, acetate, H_2, methane, and sulfate). The rate terms in each mass-balance equation include the proper stoichiometry for each catabolic reaction. For example, the net rate of H_2 production (R_{H2}, in moles of H_2 per liter per unit time) is given by:

$$RH_2 = 4rg - 2rp - 2rb + 3rpm + 2rbm - rH_2m - rH_2s$$

The first three terms on the right side indicate that the direct formation of H_2 from fermentation of glucose equals 4 times the molar fermentation rate of glucose (r_g) less 2 times each of the propionate and butyrate formation rates (r_p and r_b). The fourth and fifth terms represent the H_2 formation by the hydrogen-producing bacteria that ferment propionate (r_{pm}) and butyrate (r_{bm}). The sixth and seventh terms represent the direct H_2 consumption by the H_2-oxidizing methanogens (r_{H2m}) and sulfate reducers (r_{H2s}).

In all cases, the reaction rate term is a mixed second-order function of the Monod form,

$$r_{ij} = q_{ij}X_j \frac{S_i}{K_{ij} + S_i}$$

where r_{ij} is the rate of utilization of substrate i by microbial type j, q_{ij} is the maximum specific rate of utilization of substrate i by microbial type j, X_j is the concentration of microbial type j, S_i is the concentration of substrate i, and K_{ij} is the half-maximum-rate concentration for substrate i utilized by species j.

The net rate of growth of each microbial type is modeled with the modified growth-rate form of the Monod function,

$$\mu_j = Y_{ij}r_{ij} - b_j$$

where μ_j is the net specific growth rate of species j, Y_{ij} is the true yield for species j degrading substrate i, and b_j is the overall specific loss rate for species j.

The model also explicitly incorporates the effects of H_2 concentration on the several steps in which it is a product. High H_2 concentration inhibits the fermentation of glucose to all of its products and the fermentations of propionate and butyrate by the obligate proton reducers. The effect is modeled by making q_{ij} dependent on the H_2 concentration:

$$q_{ij} = qij'/[1 + 1898S_{H2}]$$

where q_{ij}' is the unregulated maximum specific utilization rate, S_{H2} is the aqueous-phase concentration of H_2, and $1898S_{H2}$ represents how S_{H2} modulates the intracellular ratio [NADH]/[NAD+]. Formation of propionate and butyrate from glucose, which requires NADH, is disfavored by very low S_{H2} and is represented by

$$q_{ij} = q_{ij}'/([1 + 1898S_{H2}][1 + 1/1898S_{H2}])$$

The mathematical model was developed for a "fully penetrated" biofilm, or one in which mass-transport resistances and concentration gradients are not important. The full-penetration assumption means that the biofilm was considered to be homogeneous with regard to species distribution and substrate concentrations. Thus, while the model was able to capture the major trends in terms of substrate utilization, products formation, and accumulation of the various microbial types (Raskin, 1993), it could give no insight into spatial relationships among the microorganisms and how the spatial relations may affect inter-species transfer of H_2 (or other intermediates) and may control the overall structure and function within the biofilm. The later possibility will be pursued in future research.

Improved Biofilm Reactor Design. The fixed-bed bioreactors used in the previous research have been replaced with fluidized-bed reactors (Figure 3). The fluidized-reactor design provides for more reproducible biofilm development, sampling, and microscopic observation. These reactors consist of small sections of polyethylene tubing in suspension that serve as the substratum for biofilm growth (Figure 3). The previous fixed-bed design presented problems of non-uniform biofilm development and difficult sample removal. An important consideration in the new design was compatibility with microscopic observation. Glass coverslips were previously used to capture biofilm for microscopic examination, a format that limited microscopic observation to relatively thin biofilms (Amann et al. 1992). An important advantage of the polyethylene substratum is that it can be sectioned prior to probe hybridization and microscopic examination. The polyethylene tubing sections are colonized on the interior and exterior surfaces. The inner surface thereby provides a protected surface for uniform biofilm development. Thus, biofilm samples taken from the reactor in bulk (i.e. bulk extraction of nucleic acid from multiple tubing sections) or as individual tubing sections (for microscopic examination, below) are of comparable composition.

Improved Imbedding and Sectioning Techniques for Fluorescence Microscopy. In order to visualize the spatial structure of the biofilms growing on the tubing, the samples were sectioned following paraffin embedding using a variation of standard tissue sectioning techniques used in medical histology laboratories. Paraffin embedded samples can be sectioned

at thicknesses ranging from 2 μm to approximately 10 μm thick. In addition, and with relevance to the use of in-situ hybridization, it is important that the sections be thin so that the fluorescence from out-of-focus cells does not obscure the fluorescent signal from the cells in focus. We have found 2-3 μm sections to be best.

A dialysis method was also developed for gentle fixing, concentrating and embedding of biofilm floc samples. The key methodological development was stabilization of biofilm samples in agarose prior to embedding in paraffin. The agarose does not contribute significantly to autofluorescence of samples following in situ hybridization of the DNA probes (Figure 4).

Distribution of Sulfate-reducing Bacteria in Marine Microbial Mats. During this granting period we established a collaborative association with Dr. B. Risatti (State Geological Survey) to examine the distribution and activity of sulfate-reducing bacteria a marine microbial mat community. The cyanobacterial microbial mat specimens were obtained from a saline evaporation pond of the Exportadora de Sal (Baja California) where bacterial dissimilatory reduction of sulfate is a major pathway in organic carbon mineralization as is the case for many marine systems. It has recently been shown for these well-studied mats that the highest rates of sulfate reduction coincide with the photo-oxic zone in the upper 3-4 mm of the mat in apparent contradiction to the conventional view that bacterial sulfate reduction is an obligately anaerobic process. The collaborative research served two objectives related to ONR sponsored research: 1) validation of the use of the DNA probes for sulfate-reducing bacteria in an open marine system and 2) identification of novel sulfate-reducing bacterial populations, including those associated with aerobic sulfate reduction.

Samples of the mat were collected by coring and subsampling at 2mm increments. Total RNA extracted from each slice was immobilized on nylon membranes and hybridized individually with radioactively labeled probes to quantify the individual rRNA target populations (Figure 2). This demonstrated a dramatic stratification of populations and suggested certain relationships between populations and mat processes (Risatti et al. in press). Comparison of population profiles to previously observed rates of sulfate reduction indicated the presence of additional novel populations of sulfate-reducing bacteria both within the photo-oxic zone and deeper in the mat. Thus, the DNA probes developed for our ONR sponsored research were demonstrated to be of utility in open marine systems and suggested the existence of uncharacterized sulfate-reducing bacteria.

SIGNIFICANCE:

Competition between SRB and methanogenic bacteria was demonstrated to be only partly controlled by sulfate availability. This suggested close syntrophic associations between these and other biofilm populations that were not captured by our model of the biofilm community. Improved biofilm sampling and microscopy techniques were developed to further characterize these population associations at the level of individual cells. Measurements of single cell activities revealed significant differences in activity as a function of age and position in biofilms. These results have emphasized the importance of direct structure/ function analyses, in combination with mathematical modeling, to identify key metabolic interactions among biofilm populations. In addition, this research demonstrated the feasibility of using molecular phylogenetic information as a means to both identify and isolate novel biofilm (or environmental) microbial populations. These observations and technical developments should provide for a more rational foundation to define strategies for control and manipulation of biofilms.

PUBLICATIONS, ABSTRACTS, AND INVITED PRESENTATIONS:

1. Stahl, D.A. The natural history of microorganisms. ASM News. **59**: 609-613 (1993).
2. Raskin, L., J.M. Stromley, B.E. Rittmann, and D.A. Stahl. Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens . Appl. Environ. Microbiol. **60**: 1232-1240 (1994).
3. Raskin, L., L.K. Poulsen, D.R. Noguera, B.E. Rittmann, and D.A. Stahl. Quantification of methanogenic groups in anaerobic biological reactors using oligonucleotide probe hybridizations Appl. Environ. Microbiol.. **60**: 1241-1248 (1994).
4. Korhring, L.L, D.B Ringelberg, R. Devereux, D.A. Stahl, M. Mittelman, and D.C. White. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. FEMS Micro. Lett. **119:** 303-308 (1994).
5. Stahl, D.A., R.I. Amann, L.K. Poulsen, L. Raskin, and W.C. Capman. The use of fluorescent probes for determinative microscopy. In: Archaea: A laboratory manual. F.T. Robb, K.R. Sowers, S. DasSarma, A.R. Place, H.J. Schreier, and E.M. Fleischmann (Eds.). Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y. (in press).
6. Stackebrandt, E., D.A. Stahl, and R. Devereux. Taxonomic relationships. *In* Biotechnology Handbooks: Sulfate-Reducing Bacteria. L.L. Barton (Ed.) Plenum Publishing Company. London, England (in press).
7. Raskin, L., R.I. Amann, L.K. Poulsen, B.E. Rittmann, and D.A. Stahl. Use of ribosomal RNA-based molecular probes for characterization of complex microbial communities in anaerobic biofilms (submitted to Water. Sci. Technol.)
8. Raskin, L., B.E. Rittmann, and D.A. Stahl. Molecular studies suggest direct competition between *Desulfovibrio* and *Methanobacteriales* during a long-term study of anaerobic biofilm reactors. American Society for Microbiology. Las Vegas, Nevada. May 23 - 27, 1994
9. Society for Industrial Microbiology and Canadian Society for Microbiology Joint Annual Meeting. Toronto, Canada, July 31-August 6, 1993 (**Invited Speaker).**
10. NATO Advanced Research Workshop. Structure, development, and significance of microbial mats. Arcachon, France. September 27- October 1, 1993 (**Invited Participant**).
9. European Community Meeting on Biosafety and Microbial Ecology. Granada, Spain. October 24 - 27, 1993 (**Invited Keynote Speaker).**
10. American Society for Microbiology. Las Vegas, Nevada. May 23 - 27, 1994 (**Invited Speaker**).
11. American Society for Microbiology International Conferences on Multicellular Behavior of Bacteria, Woods Hole, MA. March 28-April 1, 1993 (**Invited Participant**).
12. German Ministry for Research and Technology and the German National Research Center for Biotechnology Workshop on Molecular Microbial Ecology and Biosafety. Wolfenbüttel, Germany, June 1994 (**Invited Participant).**
13. Indiana University, March 1993 (**Invited Seminar Speaker**).
14. Microbial Diversity Mini Symposium Series. Biofilm Mini Symposium. Marine Biological Laboratories. Woods Hole, MA, June 26, 1993 (**Invited Speaker**).
17. Army Constrution Engineering Laboratory and Norwestern University Environmental Council Symposium on Frontiers in Environmental Technology. Northwestern University. June 1994 (**Invited Speaker**).
18. University of Calgary, March 1994. **Alberta Heritage Foundation for Medical Research Visiting Lecturer**
19. University of Wisconsin, February 1993 (**Invited Seminar Speaker**).

FIGURES

Figure 1. Response of SRB biofilm reactor populations to the addition or removal of sulfate as a terminal electron acceptor for anaerobic respiration. Population response was determined using the collection of group-specific DNA probes for sulfate-reducing bacteria and methanogens developed during our previous ONR sponsored research (Devereux et al. 1992, Raskin et al. 1994). The lower panel(s) in each (**A-D**) show(s) chemical profiles for each biofilm reactor [two control reactors (**A and D**) and two perturbed reactors (**B and C**)]. All four reactors had been run continuously for approximately one year prior to perturbation of two reactors (sulfate addition or sulfate removal). The remaining two reactors served as controls. Day zero corresponds to the addition (**B**) or removal (**C**) of sulfate to (or from) the nutrient feed. The percentages of desulfovibrio in the sulfate-plus biofilm reactors (**B and D**) ranged from 25 - 35%, whereas the range in the sulfate-minus reactors was 17 - 20%. The average reduction in desulfovibrio abundance was approximately 40% following removal of sulfate. This was comparable to abundance in the sulfate-minus control reactor. The evolution of measurable methane in reactor **C** corresponded to the increase in abundance of the Methanobacteriales target group (**C-top**). This target group was in least abundance, relative to the other methanogen target groups, prior to sulfate removal. This suggests that this group is less competitive at low hydrogen partial pressures (e.g. in the presence of hydrogenotrophic SRB). Their increase following the removal of sulfate suggests they may be more competitive as elevated hydrogen concentrations. This is consistent with the generally higher values for K_m (1/2 substrate saturation), μ_{max} (maximum growth rate), and q_{max} (maximum substrate uptake) for characterized representatives of this target group. Of particular note was the immediate onset of sulfate-reduction in the steady-state methanogenic reactor following sulfate addition (**B**). This is consistent with the presence of a standing population of desulfovibrio in the sulfate-minus reactor, prior to sulfate addition. However, all reactors (including the control reactors) demonstrated an immediate and transient chemical and population response following day zero. This was a consequence of repeated and frequent sampling of each biofilm reactor during the time immediately following perturbation.. Sampling alone was a perturbation to these biofilm reactor systems. This may in part have been a consequence of brief exposure to more oxidizing condition during the removal of samples. We have recently modified reactor design (Figure 3) to facilitate sampling with minimal perturbation of the system. The acetate levels in the reactor influent are also of considerable interest. Three general trends are evident: **1**) acetate levels are lowest in the sulfidogenic reactors. However, the genus generally thought to specialize in acetate oxidation (*Desulfobacter* spp.) is in low abundance in both sulfidogenic reactors; **2**) in the methanogenic reactors, the highest methane evolution corresponds to periods of low effluent acetate. However, the characterized genera of acetoclastic methanogens (*Methanosarcina* and *Methanothrix/Methanosaeta*) are not abundant in the methanogenic reactors **3**) There appears to be a consistent oscillation of effluent acetate. In the sulfate-plus biofilm reactors acetate concentrations generally appeared as a dampened oscillation with increasingly lower effluent acetate following each oscillation (peak). The oscillations observed in the methanogenic reactors were not associated with decreased effluent acetate. Of particular note was the similarity in oscillations for all reactor systems. Four acetate effluent peaks are evident in three reactors (**A, B, and D**) and three peaks in one (**C**). The periods between each peak of acetate are approximately 25-33 days, 115-150 days, and 40-90 days. Thus, these oscillations appear to be independent of sulfate input and suggests a more fundamental mechanism of control of acetate oxidation/fermentation.

Figure 2. Saline microbial mat study. Relative and absolute abundances of archaeal and different SRB target group rRNA in nucleic acid extracted from marine microbial mat samples obtained from different depths. **A.** Nanograms target organism rRNA per gram mat (a measure of absolute population size). **B.** Percent target organism rRNA (a measure of the fraction of total 16S-like rRNA detected by each group-specific probe in relation to the universal probe). Average depth is the midpoint of the depth range for a given mat section. Values from replicate samples have been averaged.

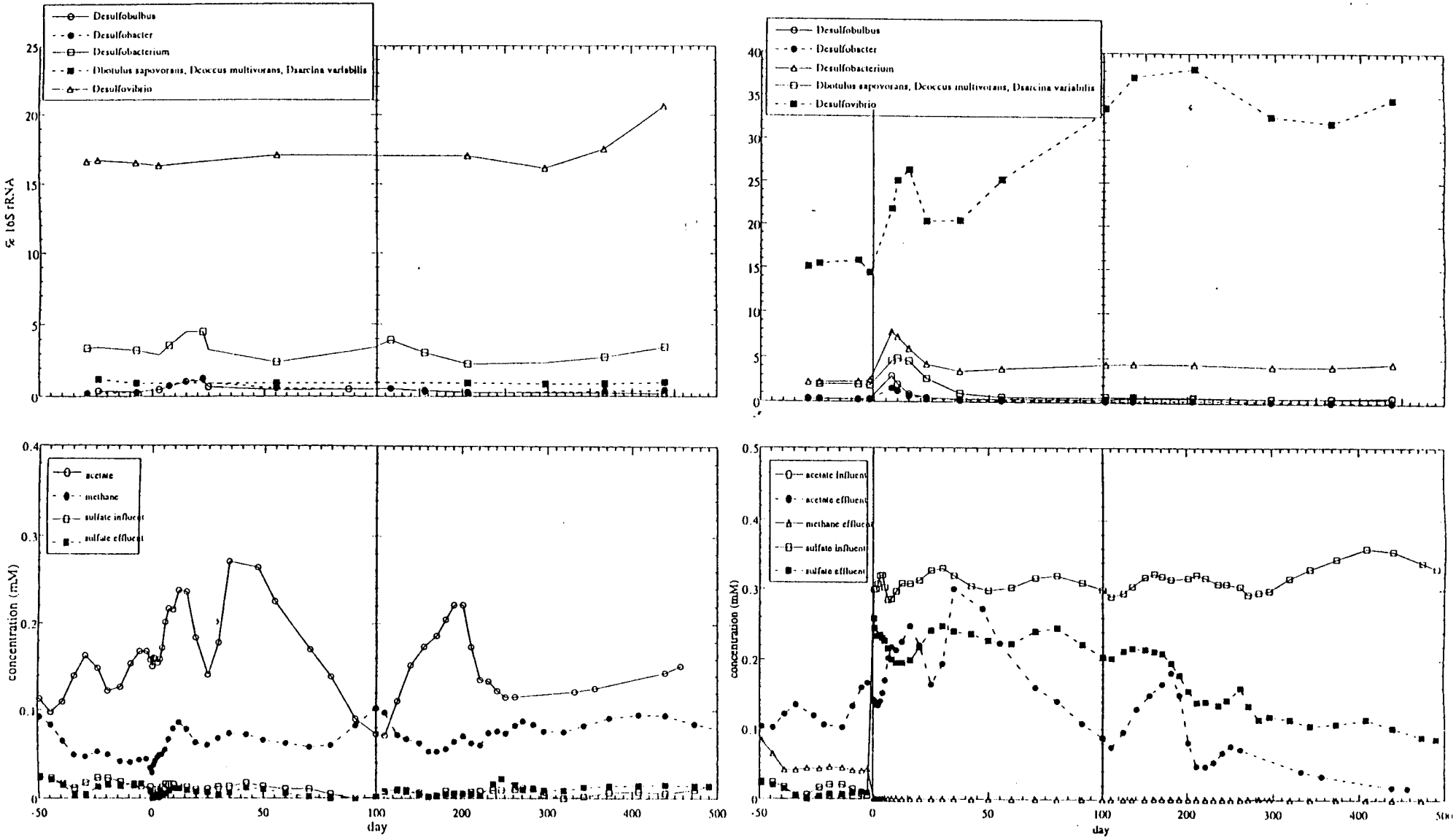
Figure 3. Improved Bioreactor Design. Fluidized bed reactor containing polyethylene tubing as substratum for biofilm attachment and growth.

Figure 4 (A - C). Three μm thick sections of biofilm/floc that grew inside a piece of polyethylene tubing (as shown in Figure 2) placed in a methanogenic anaerobic bioreactor. The entire 4 mm long piece of tubing was fixed in paraformaldehyde, embedded in paraffin, and sectioned. Following removal of paraffin using xylene, in situ hybridization was performed using a bacterial (eubacterial) probe and an archaeal (archaebacterial) probe simultaneously. **A)** Phase contrast. **B)** Fluorescein-labeled bacterial probe. **C)** Rhodamine labeled archaeal probe. **D - F)** A 2 μm thick section of a floc from a sewage enrichment, probed simultaneously with bacterial and archaeal probes. This enrichment had previously been transferred more than 12 times using medium with butyrate as the sole carbon and energy source, resulting in a simplified community consisting of methanogens (primarily *Methanosarcina*-like) and unidentified syntrophic bacteria. **D)** Phase contrast. **E)** Fluorescein labeled bacterial probe. **F)** Rhodamine labeled archaeal probe. **E1 - F1, and E2 - F2)** Enlarged views of regions from **E)** and **F)**, demonstrating great differences in fluorescent signal intensity in bacteria growing in different regions of the floc. Frame **E1** represents a 6 second camera exposure. All other frames were 2 second exposures. Of particular note is the extreme nonhomogeneous character of the spatial distribution of these two general populations.

Figure 1

A. Methanogenic control (minus sulfate)

B. Day 0 sulfate addition



C. Day 0 sulfate removal

Figure 1

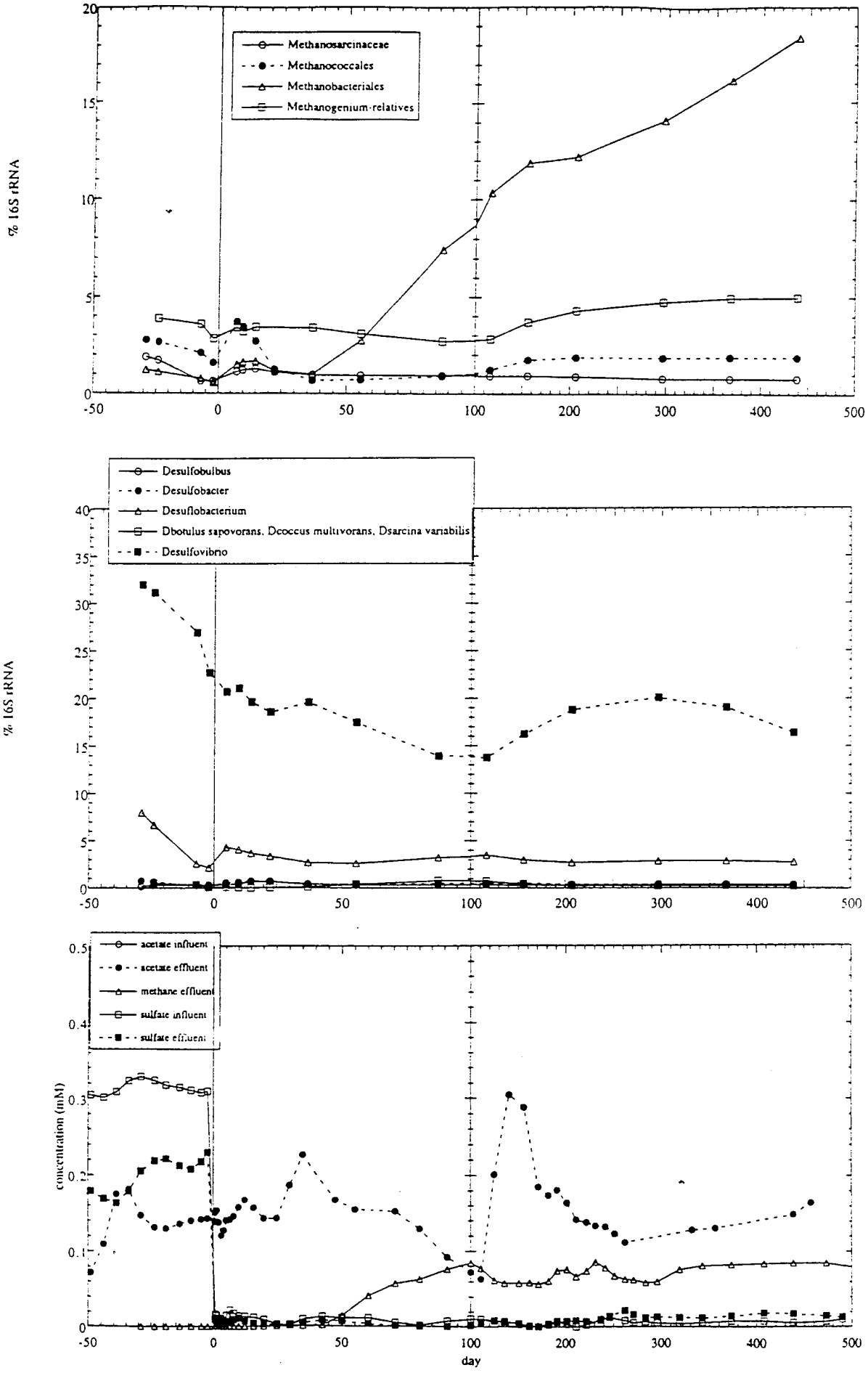


Figure 1

D. Sulfidogenic control (plus sulfate)

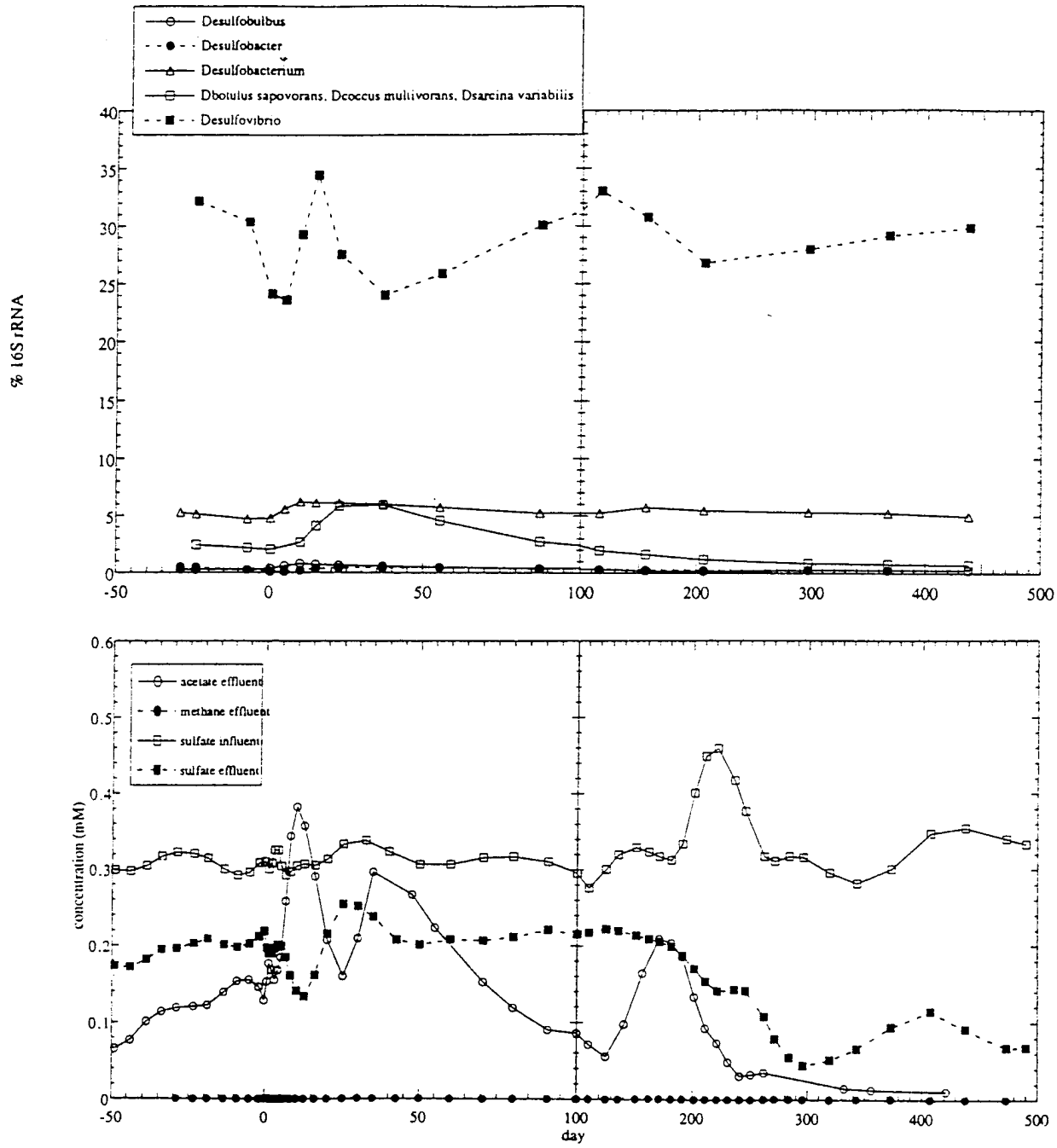


Fig. 2

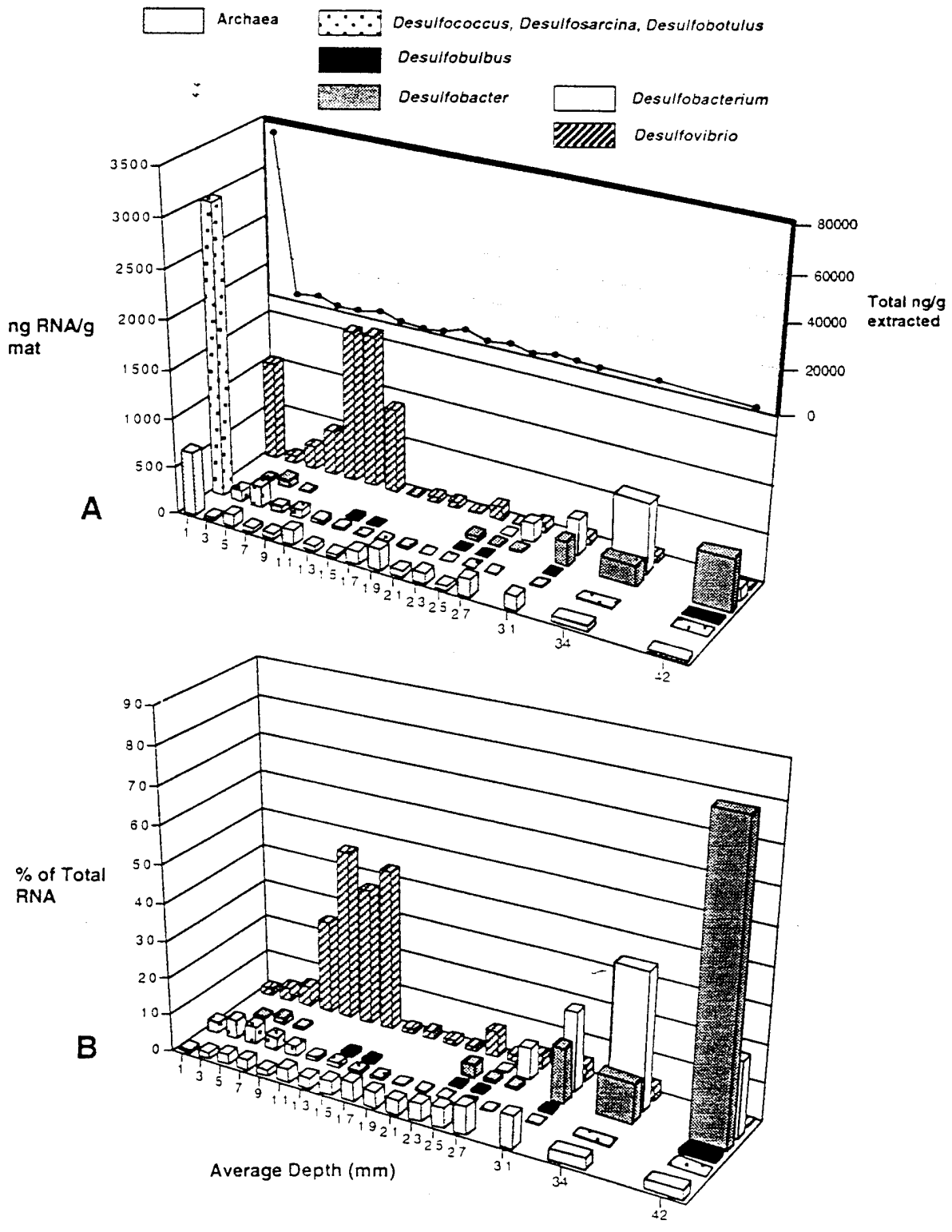
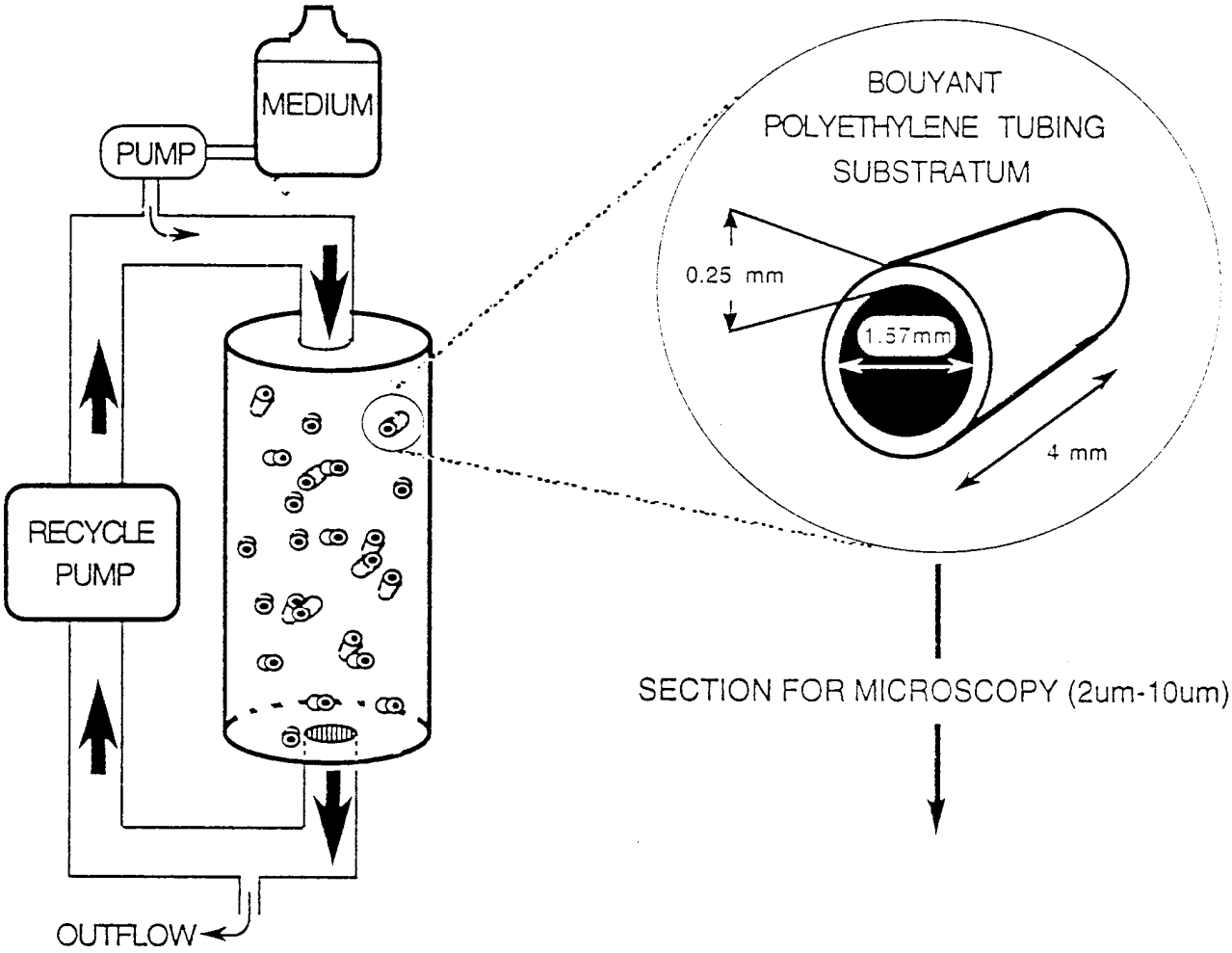
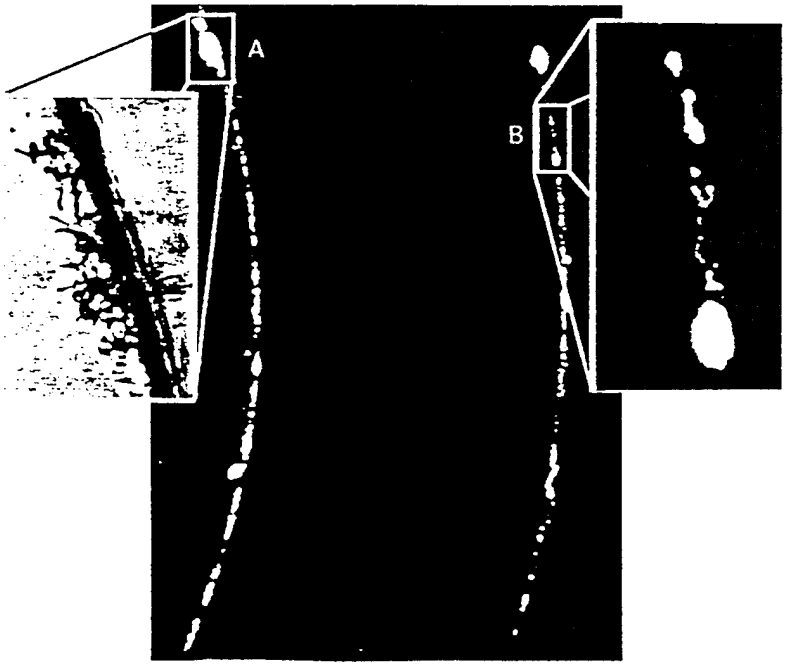

Archaea
Desulfococcus, Desulfosarcina, Desulfobotulus
Desulfobulbus
Desulfobacter
Desulfobacterium
Desulfovibrio
ng RNA/g mat
Total ng/g extracted
A
% of Total RNA
B
Average Depth (mm)

Fig. 3

FLUIDIZED BED BIOFILM REACTOR





DAPI STAINED SECTION
A) Phase Contrast
B) Fluorescence

Fig. 4